The cells were cultured with only GM and GM+C3 inhibitor. Then the number of cells was counted.
Results: The inhibition of HGC proliferation induced by GM was strongly found in time course and a dose dependent manners. In agar electrophoresis(EP), DNA extracts showed a negligible EP pattern. In the TUNEL the cell cultured with GM showed a decrease of total cell number(CN)and increase of positive CN. In IHC staining in P53, ss-DNA, bcl2 and C3 of GM treated cell culture (CC) for 24 hours, total CN decreased and positive cells increased. In the absence of GM, positive cells were scattered. The cell growth inhibition by GM was almost blocked by C3 inhibitor (Table 1).
Conclusions: The EP pattern often is seen in adhesive cells. The purpose of this study was to verify that the cell death is AP, not to investigate the activation rout of AP. To verify that GM causes the AP of cultured cells, it must be confirmed that there is a decrease in the cell count after the addition of GM on CC and that there is inhibition of a decrease in the cell count after the addition of GM+C3 inhibitor on CC. In this study AP was ascertained (Table 1). It was found that GM induced AP not necrosis of HGC. However,as HGC belongs to Type II cell, we speculate the following rout:P53 activation->BH3 activation $\rightarrow$ release of cytochrome c from mitochondria $\rightarrow$ Apf1 activation $\rightarrow$ C3 activation. TUNEL and IHCS showed the staining pattern supported AP. Perhaps, we think that when AP-promoting protein is more predominant than AP-inhibiting protein in the AP rout, AP may be induced. GM will be hoped as a good drug (perhaps to TAE & IV) that attacks to HCC by AP only with a little bit of side effects and without the effect to normal liver.

Table1. The effects of GM+C3 inhibitor on the proliferation of HGC

No.	Culture method	Total cell count
1	HGC for 24 hours culture	26.58×10^5
2	As 1 w/ GM 1,000 μM	18.46×10^5
3	As 2 + C3 inhibitor 80 μM	23.70×10^5 cells/ml

1029 POSTER

P38 MAPK − a Potential Target for Metastatic Melanoma Therapy?

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Background: Metastatic melanoma (MM) is the most malignant of all skin cancers. Only a minority of patients respond to standard therapy, so the perspective for more efficient treatments lies in the development of new strategies for a selective inhibition of growth and elimination of MM cells. Overactivation of the ERK-MAPK signaling is frequently found in MM and is usually caused by mutations of Ras, B-Raf or PTEN. In contrast, the role of the p38 MAPK pathway in MM development and survival is poorly understood.
We have shown previously, that a small molecular inhibitor of p38 α/β MAPK SB202190 (SB) selectively induces autophagy in MM cell lines, but not in other cell types we tested. In our previous experiments, we have demonstrated that SB-induced autophagy promotes the survival of MM cells and combining SB with autophagy inhibitors can lead to a significant decrease of MM cell viability.
Material and Methods: Human melanoma cell lines A375, RVH-421, human osteosarcoma cells U2OS and human foreskin fibroblasts SCRC 1040 were cultured in sterile conditions. Light microscopy of living cells and viability assay with flowcytometry were performed after 24/48-hour of cultivation in the presence of selected inhibitors. Levels of antiapoptotic proteins were analyzed by western blotting.
Results: We investigated the effect of the BH3 mimetic gossypol on MM cell viability as a tool for maximizing the cytotoxic effect of SB and its combinations with autophagy inhibitors. In A375 cells this combination leads to the apoptotic death of nearly 90% of cells. Interestingly, the co-treatment with gossypol is sufficient for efficient induction of apoptosis in SB-treated A375 cells without the need for autophagy inhibitors. In contrast, in RVH-421 cells the addition of gossypol does not enhance the cytotoxicity caused by SB in combination with autophagy inhibitors. Interestingly, SB inhibits expression of Mcl-1 in RVH-421 but not in A375 cells and this might be the reason why further sensitization with gossypol is required for the triggering of cell death in A375 cells.
Conclusions: We showed that SB acts as a cellular stress inducer in MM cells to which they respond by inducing pro-survival autophagy. Our results indicate that increased expression of Mcl-1 protein may be a factor involved in the MM cells resistance to SB treatment and this resistance could be overcome with small molecule inhibitors such as gossypol.
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1030 POSTER

Re-expression of p16 Mediates Apoptosis in Cholangiocarcinoma With Low Rb Level

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Background: Cholangiocarcinoma (CCA) is a malignant bile duct epithelium which is a major liver cancer found in Northeast Thailand. The mortality rate of CCA is high while the survival rate is poor which results from advance stage of the patient at initial diagnosis. The tumour suppressor gene *p16* is a member of the INK4 family of cyclin dependent kinase inhibitor. It functions by direct binding to Cdk4/6 and preventing the phosphorylation of Retinoblastoma (Rb), which in turn blocks cell cycle transition from G1 to S phase. Our previous study showed that loss of p16 protein expression is the most frequent event in CCA (81.5%) and is significantly associated with poor survival. The present study aimed to address the role of re-expression of p16 in CCA cell lines harboring different levels of Rb using adenovirus system.
Materials and Methods: CCA cell lines KKU-100, M055, and M139 established from intrahepatic CCA samples were used in this study. These cell lines expressing no endogenous p16 were infected with p16 recombinant adenovirus vectors (*Ad-p16*) to mediate exogenous expression of p16. The Ad5CMV-Luc vector encoding luciferase was used as a control. Cell cycle and apoptosis were determined by Flow cytometry while beta-galactosidase associated senescence was performed using the X-gal staining method. Subcellular localization and protein levels of p16 and Rb were assayed using immunocytochemistry and Western blotting, respectively. *Rb* knockdown was performed using small interfering RNA (siRNA).
Results: Infection with *Ad-p16* resulted in significantly high level of p16 expression in all CCA cell lines. Exogenous p16 mediated senescence in M055 and M139 cells expressing high level of Rb while KKU-100 which expresses low level of Rb was undergone apoptosis. Apoptosis was observed in *Rb* knockdown M055 and M139 cells infected with *Ad-p16*.
Conclusions: Re-expression of p16 is capable of mediating apoptosis in CCA cell lines through low level of Rb expression and *Ad-p16* may be a promising candidate for cancer gene therapy in CCA.

1031 POSTER

Protein-bound Polysaccharide From Phellinus Linteus Inhibits Tumour Growth, Invasion, and Angiogenesis Through Inhibition of Wnt/β-catenin Signaling in SW480 Human Colon Cancer Cells

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Background: Polysaccharides extracted from the *Phellinus linteus* (PL) mushroom are known to possess anti-tumour effects. However, the molecular mechanisms responsible for the anti-tumour properties of PL remain to be explored. In this study, the anti-cancer effects of PL were examined in SW480 colon cancer cells by evaluating cell proliferation, invasion and matrix metallo-proteinase (MMP) activity.
Background: Polysaccharides extracted from the *Phellinus linteus* (PL) mushroom are known to possess anti-tumour effects. However, the molecular mechanisms responsible for the anti-tumour properties of PL remain to be explored. In this study, the anti-cancer effects of PL were examined in SW480 colon cancer cells by evaluating cell proliferation, invasion and matrix metallo-proteinase (MMP) activity.
Material and metods: The anti-angiogenic effects of PL were examined by assessing human umbilical vein endothelial cell (HUVEC) proliferation and capillary tube formation. The *in vivo* effect of PL was evaluated in an athymic nude mouse SW480 tumour xenograft model.
Results: PL (125–1000 μg/ml) significantly inhibited cell proliferation and decreased β-catenin expression in SW480 cells. Expression of *cyclin D1*, one of the downstream-regulated genes of β-catenin, and T-cell factor/lymphocyte enhancer binding factor (TCF/LEF) transcription activity were also significantly reduced by PL treatment. PL inhibited *in vitro* invasion and motility as well as the activity of MMP-9. In addition, PL treatment inhibited HUVEC proliferation and capillary tube formation. Tumour growth of SW480 cells implanted into nude mice was significantly decreased as a consequence of PL treatment, and tumour tissues from treated animals showed an increase in the apoptotic index and a decrease in β-catenin expression. Moreover, the proliferation index and microvessel density were significantly decreased.